RESPIRATORY STUDIES OF SINGLE CELLS. II. OBSERVATIONS ON THE OXYGEN CONSUMPTION IN SINGLE PROTOZOANS [1]

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During the development of the reference diver technique (Scholander, Claff and Sveinsson, 1952) for studying single cell respiration, many measurements were made on single protozoans. We are here presenting observations on (1) the relation between cell volume and respiratory rate as determined over a thousandfold range in size, using individual cells from three species of protozoans, and (2) the metabolic change occurring at excystment and encystment of *Bresslaua*.

Material and Methods

Respiratory measurements

A single animal was pipetted from the stock culture, usually hay tea with a few wheat grains, and transferred with a minimum amount of water into a dish containing sterile Ringer solution diluted 50 times. Two to three additional transfers were made, where the cell was allowed to swim through, or was moved through, relatively enormous amounts of sterile solution. If any bacteria were left clinging to the cell the inorganic medium would hardly support any appreciable growth during the time of the experiment. All measurements were made at 25° C. and each run lasted 60–70 minutes. Our runs confirm the findings by Amberson (1928) that the oxygen rate stays constant in spite of large changes in the oxygen tension. After the run the cell was transferred to a dish and the cell volume could subsequently be measured.

Measurement of cell volume (Fig. 1, A and B)

The cell volumes were measured by squeezing the cells between two glass surfaces spaced at a known distance, and tracing the surface contour. Mast and Fowler (1935) and Pèterfi and Maleci (1938) used a similar method of deforming the cells, by sucking them into a capillary tubing of known diameter and measuring the length.

For the measurements two brass wire frames 1 × 2 cm. are prepared. Stretched across the frame on the under side are two platinum filaments [2] of accurately known diameter. One frame had filaments of 19 μ, the other of 38 μ. A 10 × 5 mm. piece of a microscope slide serves as a thick and heavy coverslip. The frame is

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[2] Obtained from Baker and Co., Newark, N. J.

placed on a microscope slide, and the cell in a small drop of water is placed between the filaments. The cover glass is carefully put on. Dust must be carefully avoided on the spacer filaments. A paramecium can move about slowly even when squeezed flat to about one fifth or one sixth of its normal diameter. When at rest its circumference is rapidly traced through an Abbé prism onto a piece of paper which has a known weight per surface area. The cell tracing is cut out and weighed and the cell volume is calculated from the surface area and the thickness. In one

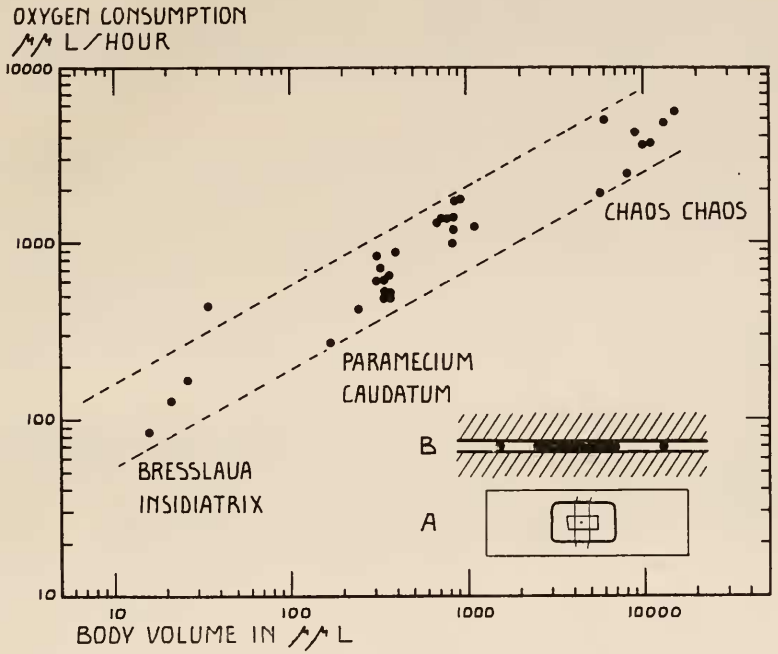

FIGURE 1. Ratio of oxygen consumption to cell volume, both determined in the same individual cell. The parallel lines indicate the general trend of the data. The slope of the lines is the exponent n of the equation $M = K \times W^n$, where M is the oxygen consumption, K is a proportionality constant and W is the weight. We attach no significance to the empirical value of n fitting these data. Insert A. Method for measuring cell volume by squeezing cell to a known thickness under a cover glass resting on two spacing filaments. Insert B. Cross section showing spacing filaments and flattened cell between the glass surfaces. One $\mu\mu$l = one millionth of one mm^3.

species of *Paramecium* several measurements on the same individuals gave an agreement within ± 6%.

The amoeba *Chaos chaos*, which cannot be flattened nearly as much as a paramecium without rupturing, was squeezed between thicker filaments or even under the coverslip of a blood cell counting chamber. In order to succeed in the measurement it is necessary to tease the amoeba to contract by tapping the glass and trace it quickly before the protoplasm begins to flow out at the edges. In the amoeba the volume measurements are probably not better than ± 10%.

Relation of Size to Oxygen Consumption in Some Protozoans

Using the reference diver technique we have determined the oxygen consumption in a series of cells of different species and size. The absolute accuracies obtained are estimated to be within about ± 10% for the smaller species and ± 5% for the larger species.

There seem to be very few cases where metabolic rate and cell volume are given for the same cell. Holter and Zeuthen (1948) give such data for amoebae (*Chaos chaos*), with the respiration measured by the Cartesian diver technique, and the cell volume determined colorimetrically (Holter, 1945), or estimated from the buoyancy of the cell in water (Zeuthen, 1948). In another amoeba, *Difflugia* sp., the oxygen consumption was determined individually in seven animals and the average size is given (Zeuthen, 1943).

There are many observations available where the average oxygen consumption per individual can be calculated from estimation of the number of cells used in mass runs, and where the average cell volume can be estimated from linear measurements or a hematocrit technique. These runs have often yielded extremely variable results. The figures given for one of the most studied protozoans, *Paramecium caudatum,* range from 120 $\mu\mu$l/hour/cell to 5600 $\mu\mu$l (*cp.* compilations by von Brand, 1935, and Jahn, 1941). These very large discrepancies are sometimes due to inadequate methods. In many cases bacterial infection may have been a large source of error.

Especially in mammals it has been well established that the resting metabolic rate (M) can be described as proportional to an exponential function of the weight (W), according to $M = K \times W^n$. The exponent n has been found empirically to be near 0.75 (see Brody, 1945; Kleiber, 1947). There are many known exceptions to this rule (see Scholander, Hock, Walters and Irving, 1950). A great deal of similar information has also been collected for poikilotherms (see Zeuthen, 1947; Hemmingsen, 1950). When the metabolic rate and the weight are presented on a double log plot the exponent will appear as the slope of the line of correlation.

In Figure 1 we have accordingly plotted the oxygen consumption versus the volume of the cell, and in this material of a thousandfold range in size, the exponent (slope) is low, about 0.55. If the dotted parallel lines are extended down to the size of bacteria, we will hit *Bacterium coli* at a cell volume of 0.5–1 μ^3 and an O_2 consumption of 0.1–1 $\mu\mu$l/cell/hour (Martin, 1932; Huntington and Winslow, 1937; Hershey and Bronfenbrenner, 1938), but we will be about ten times too high for *Photobacterium phosphorescens,* which, with a volume of 1.7 μ^3, has only 0.06 $\mu\mu$l O_2 consumption (Harvey, 1928). If we start at the upper end, the data on *Chaos chaos* by Holter and Zeuthen (1948) fit well between the lines and so do the data on *Difflugia* when the shell is disregarded (Zeuthen, 1943). The oxygen consumption of *Tetrahymena geleii,* with 15–24 $\mu\mu$l cell volume, has been found by several investigators to lie between the lines (see Ormsbee, 1942; Pace and Lyman, 1947), but it may rise up to three to four times higher than the upper line when given suitable substrate. *Astasia klebsii,* on the other hand (Pringsheim, 1936; von Dach, 1940, 1942), was found about twenty times lower than the *"coli-chaos"* line when starved, but on the line when fed on acetate (cell volume about 5 $\mu\mu$l, O_2 consumption 2–4 $\mu\mu$l/hour). *Sarcina,* with a cell volume of near 2 μ^3, was found by

Gerard and Falk (1931) to use about 0.007 $\mu\mu$l/cell/hour, which places it about 100 times below the lines.

It seems that these samples of data from careful investigations point towards a very considerable variation in the relation between size and metabolic rate in the range of single cell organisms. It would appear impossible to fit the data into any simple rule, such as the "mouse to elephant" curve (Benedict, 1938), or even the "beech tree to egg of silkworm" curve discussed by Hemmingsen (1950). When interpreting the available data on single cells this author arrives at an exponent near 1, whereas according to our data and compilation many forms would fit a much lower exponent. The difficulty undoubtedly lies in the fact that we can only very poorly define what we are correlating. Although on a volume or weight basis a large number of more or less "standard" cold-blooded organisms do fit a certain exponential line, it cannot be expected that the same relation will hold also for forms that carry vast amounts of metabolically inactive material, such as corals, jellyfish, eggs, trees, etc., unless possibly some correction for the inert material could be made. In many forms (seeds, spores, etc.) it is likewise very difficult to define what is meant by resting rate. These difficulties are certainly no less in dealing with the single cell organisms.

In our series, we are comparing fast swimming, highly active *Paramecium* and *Bresslaua* with extremely sluggish amoebae, and we would not expect that both would belong on the same line unless the active ones were resting. In our material we have not seen any striking changes in O_2 consumption due to locomotion in the *Paramecium*, but we have not as yet studied it extensively, and we do not believe that the 200–300% spread in the data can be explained on this basis. To what extent locomotion or other activity influences the oxygen consumption in protozoans can undoubtedly be studied by the reference diver technique. The lines drawn in Figure 1 are purely descriptive for the material presented, and are at the present time void of any other significance.

Excystment and Encystment in Bresslaua

Bresslaua insidiatrix (Claff, Dewey and Kidder, 1941) will form a protective resting cyst if left long enough in pure water. It will start to circle and to eject a protective cyst shell in which it keeps turning for several hours until it finally stops. It can stay dried up as an undifferentiated, spherical, resting cyst for years. If this cyst is placed in hay tea it will start to differentiate and will excyst within an hour or so. A pulsating vacuole is formed, the animal starts circling inside the cyst shell and finally breaks through.

Gregg (1947) measured the effect of encystment on this organism, using three individuals simultaneously in a micro modification of the Warburg respirometer. He observed a 50% decrease in the oxygen consumption when the cells encysted. The rate of oxygen consumption of the free swimming cell was found to be about 10,000 $\mu\mu$l/cell/hour at a cell volume which, estimated from the linear dimensions given, cannot have been more than 500 $\mu\mu$l. This gives about ten times higher rate than indicated by the present method.

The respiratory events at excystment and encystment were followed in the respirometer, using single cysts or cells (Fig. 2). The O_2 consumption in resting

cysts was immeasurable by this method. In order to effect excystment the cyst was placed for five minutes in sterile hay tea and then transferred several times in sterile 1/50 dilute Ringer solution in which it was run. Hay tea is a better medium for excystment but was found too difficult to maintain sterile.

After the activation by hay tea there is a steady increase in oxygen consumption, and at the moment the organism breaks through the cyst there is a definite but often quite small rise. It is conceivable that this rise is merely due to the elimina-

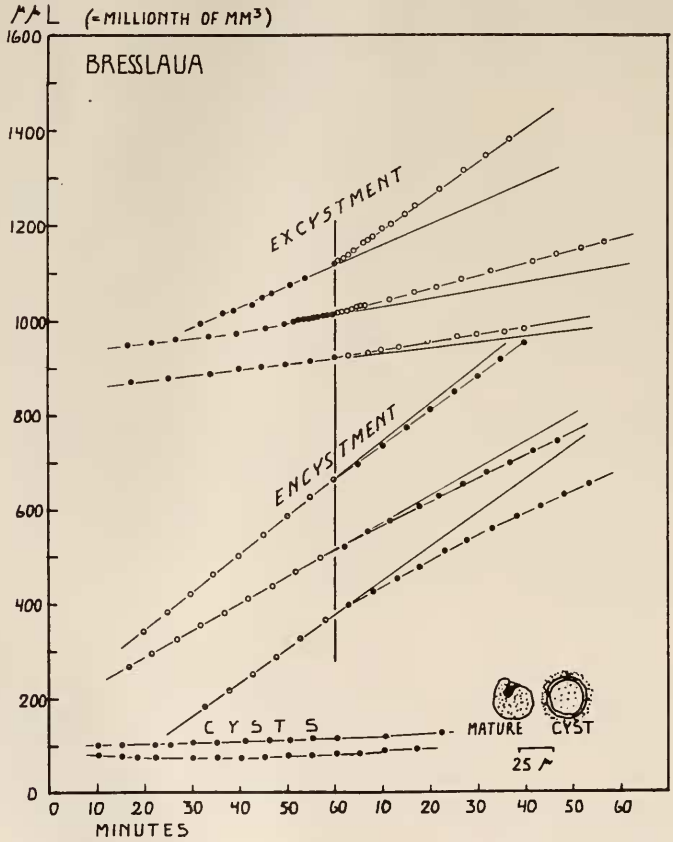

FIGURE 2. Oxygen consumption at excystment and encystment in single individuals of *Bresslaua insidiatrix*.

tion of the shell, which would act as a limiting factor for the oxygen diffusion at the high metabolic rate of the fully developed cell.

In Figure 2 three runs are given showing the reverse effect on the oxygen consumption when the cells are encysting.

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Summary

1. Observations are reported on the oxygen consumption of single individuals of four species of protozoan using the reference diver technique.

2. The relation of oxygen consumption to cell volume is given for a series of individual protozoans covering a thousandfold range in size, and the change in oxygen consumption following excystment and encystment in *Bresslaua* has been recorded.

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